FLOW CYTOMETRY SHARED RESOURCE

STANDARD OPERATING PROCEDURE			
BD FACSDiva Software – Use, Operation, and Data Acquisition			
SOP#	Version #	Category	Effective Date
IS001	1.2	Software	09-25-2022

Approved By:

Director of Flow Cytometry Shared Resource	**DATE**
Eric D Wieder, PhD. Signature: Eric Wieder	09-25-2022

SOP Revision History:

Version	Description	Effective Date	Revised By	Approved By
1.0	Development of SOP	10-09-2019	nward	Dr. Gray
1.1	Review/update	02-16-2022	pguevara	Dr. Wieder
1.2	Review	09-25-2022	pguevara	Dr. Wieder

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PURPOSE

The purpose of this standard operating procedure is to describe the procedures used by the University of Miami, Miller School of Medicine Sylvester Comprehensive Cancer Center Flow Cytometry Shared Resource staff in the use, operation, and data acquisition of the BD FACSDiva software.

SCOPE

This standard operating procedure applies to the use of the BD FACSDiva software by SCCC Flow Cytometry Shared Resource Facility personnel and users of the Flow Cytometry Shared Resource Facility.

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1. AUTHORITY AND RESPONSIBILITY

1.1 The UM SCCC Flow Cytometry Shared Resource has the authority to establish these procedures and is in control of the Standard Operating Procedure (SOP) document.

1.2 The UM SCCC Flow Cytometry Shared Resource is responsible for the implementation of these procedures and ensuring facility staff and users are appropriately trained.

1.3 The users of the UM SCCC Flow Cytometry Shared Resource are responsible for understanding and following the regulations and guidelines set forth by the core facility with regards to instrument operation and biosafety procedures.

2. TARGET USERS

2.1 UM SCCC Flow Cytometry Shared Resource staff.

2.2 Internal (university members) and external (academic and commercial) users of the UM SCCC Flow Cytometry Shared Resource BD LSRFortessa-HTS, FACSymphony A5 SE, and FACSCanto II.

3. DEFINITIONS

3.1 Browser: List folders, experiments, and experimental elements in a hierarchical view.

3.2 Cytometer window: Shows the status of the cytometer. When connected to a cytometer, the window will show status messages. With an open experiment and current tube selected, the window will display current cytometer setting and laser controls.

3.3 Inspector window: Allows viewing or modification of one or more objects in the worksheet or Browser. The contents of this window will change depending on is selected in the experiment.

3.4 Acquisition Dashboard: Comprises of controls for setting up, starting, and monitoring date acquisition and recording.

3.5 Worksheet window: Allows the creation of global or normal worksheets containing plots, gates, and statistics.

3.6 Global Worksheet: Show data for only one tube at a time. These worksheets are indicated by green tabs.

3.7 Normal Worksheet: These worksheets are tube specific.

3.8 SIP: sample injection port

3.9 FSC: Forward scatter is the measurement of cells based on size.

3.10 SSC: Side scatter is the measurement of cells based on granularity or internal complexity.

4. COMPUTER AND SOFTWARE LOGIN

4.1 To log into instrument, reservation must be first initiated through the kiosk (see SOP# GA001: iLab – Registration, Scheduling Reservations and Kiosk). Once reservation is started, the workstation monitor associated with instrument will power on.

4.2 In the BD FACSDiva Software, scroll down through the list of users to locate lab's account. The associated password will be the lab name.

5. CREATING WORKSPACE AND EXPERIMENTS

5.1 Once in BD FACSDiva Software, make sure that all necessary windows are visible: Browser, Cytometer, Inspector, Worksheet, and Acquisition Dashboard.

5.1.1 If not, under "View" the correct windows can be selected.

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5.2 Locate folder associated with corresponding user in the Browser window. If no folder is associated, add new folder either by selecting Experiment > New Folder or clicking the folder icon in the Browser window.

5.3 To add an experiment to the folder, make sure folder is highlighted then select Experiment > New Experiment > Blank Experiment or click the notebook icon in the Browser window.

5.3.1 A generic name will be populated once experiment is created. The name can be changed by highlighting the experiment, selecting “Rename” by right clicking on the experiment, or in the Inspector window.

5.3.1.1 Rename the experiment with a descriptive title including the date to easily locate or identify experiment later.

5.3.1.2 On the FACSymphony A5 SE, prior to adding a specimen, highlight “Cytometer Settings” and right click. Select “Applications Setting” then “Apply”. A window will pop-up with the list of application settings available, select the most recent application settings and apply. This will update the voltages based on the previously performed voltration and the current CST performance check.

5.4 Add a specimen to the experiment either by selecting Experiment > New Specimen or clicking the syringe icon in the Browser window.

5.4.1 Clicking “+” on the left of added specimen will allow tubes to be viewed.

5.4.2 Additional tubes can be added by selecting Experiment > New Tube or clicking the tube icon in the Browser window.

5.5 In the Browser window, click on the arrow to the left of “Tube_001”. Arrow will turn green once tube is selected.

5.6 In the Cytometer window, select the “Parameters” tab to select fluorophores/colors used in the experiments. Delete fluorophores or colors that will not be used in the experiment.

5.6.1 Fluorophores/colors can be selected using the scroll down menu and added by clicking the “Add” button.

5.6.2 In the case that only one fluorophore/color is being used, select the one color and delete others. No compensation controls need to be done.

5.6.3 Based on preference, FSC and SSC can be adjusted to linear or log scaling by clicking the log box in the Parameters tab.

5.6.4 Height and width scaling can be selected to aid in doublet discrimination.

5.6.5 On the FACSymphony A5 SE, you will need to decide which workflow will be used for your experiment: compensation or spectral unmixing workflow. For the compensation workflow, you will remove any detectors not used in your panel. For the spectral unmixing workflow, you will use all 48 detectors regardless of your panel.

5.7 Once correct fluorophores/colors have been selected, create compensation

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controls under Experiments > Compensation Setup > Create Compensation Controls.

5.7.1 "Create Compensation Controls" confirmation window will appear with chosen fluorophores/colors. Double-check selected parameters and click "OK" if correct.

5.7.1 The Global Worksheet will change to Normal Worksheet automatically.

5.8 A new specimen under the Browser window named "Compensation Controls" will be listed.

5.8.1 Clicking "+" on the left next to added specimen will allow tubes to be viewed.

5.9 Select "Unstained Control" by clicking on the arrow left of the tube name and place corresponding tube on the SIP. On Acquisition Dashboard click "Acquire" to start acquisition of sample.

5.9.1 Instrument should be on run in the lowest setting.

5.10 Adjust FSC-A and SSC-A voltages in Cytometer window to place population of interest on scale.

5.11 Pre-set "P1" gate needs to be adjusted to visualize population of interest when it is within desired range.

5.11.1 Right click on the P1 gate and select "Apply to All Compensation Controls".

5.12 Double check each single-color control to make sure positive population is on scale before recording data and calculating compensation.

5.12.1 Based on marker and fluorophore, voltage of laser may need to be adjusted to place fluorescence on scale.

5.12.2 Using the spectral unmixing workflow on the FACSymphony A5 SE, voltages should not be adjusted.

5.13 Click "Acquire Data", wait a few seconds until the threshold rate is stable, and then click "Record Data".

5.13.1 Record data for all tubes in the Compensation Control specimen

5.13.2 Adjust P1 and P2 gates as needed.

5.14 Calculate compensation by selecting Experiment > Compensation Setup > Calculate Compensation.

5.15 The software will calculate compensation for all parameters. If there are no errors, click "Link & Save" in the Single Stained Setup window.

5.15.1 If there are compensation errors, go through single-color controls to make sure gates are properly adjusted.

5.15.2 Depending on the percentage of overlap or spillover, voltages on single-color controls may need to be adjusted.

5.16 Switch back to global worksheet by clicking on the top left page icon, this will allow for the collection of data for samples.

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6. WORKING WITH EXPERIMENTS

6.1 Create a FSC-A vs SSC-A plot on the global worksheet by selecting the "Dot Plot" icon on the worksheet toolbar and click one in the upper corner of the worksheet. A default size plot will be drawn.

6.1.1 Parameters on the plot can be changed by left clicking on the axis title and selecting one from the pop-up menu.

6.1.2 Size of the graph can be changed by clicking on graph perimeters and dragging.

6.2 Create a gate of choice around population of interest from the gating selections on the worksheet toolbar.

6.3 For doublet discrimination, create FSC-W vs FSC-A and SSC-W vs SSC-A scatter plots.

6.3.1 The height parameter can also be used in doublet discrimination.

6.4 Display the population hierarchy by selecting Population > Show Population Hierarchy or "Ctrl+G".

6.4.1 Additionally, the population hierarchy can be displayed by selecting a graph on the worksheet and right-clicking.

6.4.2 Population hierarchies allows visualization of defined populations and displays the relationship between different gated populations.

6.5 Create a P2 gate on the FSC-W vs FSC-A scatter plot by right-clicking on the plot > Show Populations > P1. Next, click on P1 on the population hierarchy and then selecting gate of choice for main population excluding outliers. This new population will be designated P2.

6.6 In the same manner, create a P3 gate on the SSC-W vs SSC-A scatter plot from the P2 population.

6.7 Final P3 gate will contain live and singlet population that can be further analyzed based on other fluorophores/colors.

6.7.1 Create as many dot plots or histograms as needed to evaluate data, following the previously mentioned gating techniques.

7. OTHER EXPERIMENTAL FUNCTIONS

7.1 Labeling Parameters in an Experiment

7.1.1 When a tube is selected, parameter labels can be changed in the Inspector window under the Labels tab.

7.1.2 Parameters can additionally be labeled by selecting Experiment > Experiment Layout.

7.2 Setting Up Number of Events to Record

7.2.1 In the Acquisition Dashboard, events to record can be changed under the "Acquisition Setup" section. The stopping gate can also be changed under this section.

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7.2.2 The number of events to record can also be changed by selecting Experiment > Experiment Layout > Acquisition.

7.3 Exporting an Experiment as a Template

7.3.1 Experiment templates include specimens, tubes, cytometer settings, labels, worksheet elements, and worksheets but not recorded data. Any experiment can be saved as a template.

7.3.2 Select experiment in the Browser. Choose File > Export > Experiment Template. The Export Experiment Template Wizard command box will appear.

7.3.3 Enter template Type and name for the analysis so it can be identified later.

7.3.3.1 If nothing is entered in template type, experimental template will be saved under general.

7.3.3.2 Click Next to view remaining screens and enter any detailed information that is desired.

7.4 Creating an Experiment from a Template

7.4.1 Select the folder where the experiment will be placed then choose Experiment > New Experiment. The Experiment Templates command box will appear.

7.4.1.1 Use the menu command rather than the New Experiment button when wanting to use an experiment template.

7.4.2 Locate the experimental template in the given tabs. Click OK to create the new experiment.

7.4.2.1 If nothing is entered in to “Type” for template (Refer to section 7.3.3), the experimental template will be listed under the “General” tab.

7.5 Importing an Experiment

7.5.1 Select the folder in the Browser where experiment will be imported. Choose File > Import > Experiments.

7.5.1.1 Imported experiments are different from experimental templates because they contain recorded data.

7.5.2 Locate folder that contains the experiment, select folder, click once (do not double-click) and click Import.

8. EXPORTING RECORDED DATA

8.1 Recorded data can be exported as an Experiment and/or FCS files.

8.2 Right click on the name of the experiment, then click Export > Experiments or FCS files

8.3 When exporting recorded data, either as an Experiment or FCS files, a window will appear to select the destination of the exported data.

8.3.1 If the pathway in the directory is incorrect, click on “Browse” to choose correct location where recorded data should be stored.

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8.4 For exporting recorded data as an Experiment, once the correct destination is selected, click OK.

8.5 When exporting recorded data as FCS files, after selecting to export as FCS files, a window will appear to select file version. Make sure when exporting to select “FCS 3.0 files”, then click OK

8.5.1 In the next window, click on “Browse” to choose correct location then “Save” once the correction location has been selected.

9. BATCH ANALYSIS

9.1 Performing a batch analysis gives the option to print multiple worksheets or save them as a PDF file. In addition, it will allow the exporting of the data from the “Statistics View” to a CSV file that can be later read in Excel.

9.2 To initiate a batch analysis, right click on the experiment name, specimen, or selected tubes and click Batch Analysis.

9.3 The Batch Analysis window will open that allows for the selection of software options.

9.3.1 It is important to specify the location of the save files. Click on the “Browse” button for both PDF and Export Filename to select correct location.

9.4 Once destination is selected, click Start to begin batch analysis process.

10. LOGGING OUT OF SOFTWARE

10.1 When done operating the software, select “Log Out” form the File menu. The blue log-in screen will appear.

10.1.1 Only select “Quit” in the software if you are the final user and turning the machine off.

10.1.2 Prematurely selecting “Quit” in the software can potentially cause cytometer disconnection issues when restarting the software.

10.2 Logout from the Kiosk to end your reservation and power off the workstation monitors.

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